JUN 12 1990

256143
RECORD NO.

105501
SHAUGHNESSEY NO

REVIEW NO.

EEB REVIEW

DATE: IN 12/08/89 OUT JUN 12 1990

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PETITION OR EXP. NO.

DATE OF SUBMISSION 04/28/89

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TYPE PRODUCT(S) Herbicide

DATA ACCESSION NO(S)

PRODUCT MANAGER, NO. Edwards(74)

PRODUCT NAME(S) Tebuthiuron

COMPANY NAME Eli Lilly Company

SUBMISSION PURPOSE Review Tier II phytotoxicity data and need for Tier III data.

SHAUGHNESSEY NO.	CHEMICAL	% A.I.
105501	Tebuthiuron	99.08

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 12 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Aquatic Plant Data for Tebuthiuron

FROM: James W. Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Joanne Edwards, PM 74
Reregistration Branch
Special Review and Reregistration Division (H7508C)

The Ecological Effects Branch (EEB) has completed its review of four tebuthiuron Tier II aquatic plant growth studies submitted by Eli Lilly Company. Extrapolating from recently reviewed tebuthiuron residue monitoring studies (refer to an Environmental Fate and Ground Water Branch review dated 03-20-90), EEB has estimated that concentrations in water may reach 0.54 ppm following terrestrial applications at the maximum rate of 6 lb ai/a. This concentration exceeds the EC50's for Lemna gibba, Skeletonema costatum, and Navicula pelliculosa. Consequently, aquatic plant testing at the Tier III level will be required.

The following is a brief summary of the phytotoxicity data reviewed:

1. **CITATION:** Negilski, D.S., D.W. Grothe, and P.J. Cocke. 1989. Toxicity of Tebuthiuron to Blue-green Alga (Anabaena flos-aquae) in a Static Test System. Prepared by Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN. MRID No. 410804-01.

   CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirement for a Tier II aquatic plant test using the blue-green alga Anabaena costatum. Based on algal cell counts on day-5, the EC50 and EC25 values were 4.064 and 1.69 mg/L, respectively. With aquatic residues of 0.540 ppm, this species is **not** expected to be adversely affected by tebuthiuron use.

2. **CITATION:** Negilski, D.S. and P.J. Cocke. 1989. Toxicity of Tebuthiuron to a Marine Diatom (Skeletonema costatum) in a Static Test System. Laboratory Project No. J00389. Prepared by Lilly Research Laboratories, Greenfield, IN. MRID No. 410804-02.

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirement for a Tier II aquatic plant test using the marine diatom Skeletonema costatum. Based on cell counts on day 5, the EC50 and EC25 values were 0.050 and 0.031 mg/L, respectively. With the potential for aquatic residues to reach 0.540 ppm, this species is expected to be adversely affected by tebuthiuron use.

3. **CITATION:** Negilski, D.S. and P.J. Cocke. 1989. Toxicity of Tebuthiuron to a Freshwater Diatom (Navicula pelliculosa) in a Static Test System. Prepared by Lilly Research Laboratories, Greenfield, IN. MRID No. 410804-03.

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirement for a Tier II aquatic plant test for the freshwater diatom Navicula pelliculosa. Based on cell counts on day-5, the EC50 and EC25 were 0.081 and 0.035 mg/L, respectively. With the potential for aquatic residues to reach 0.540 ppm, this species is expected to be adversely affected by tebuthiuron use.

4. **CITATION:** Negilski, D.S. and P.J. Cocke. 1989. Toxicity of Tebuthiuron to Duckweed (Lemna gibba) in a Static Renewal Test System. Laboratory Project No. J00588. Prepared by Lilly Research Laboratories, Greenfield, IN. MRID No. 410804-04.

CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirement for a Tier II aquatic plant test using Lemna gibba. Based on 14 day biomass, the EC50 and EC25 values were 0.135 and 0.066 mg/L, respectively. With a potential for aquatic residues to reach 0.540 ppm, this species is expected to be adversely affected by tebuthiuron use.

F16

MRID No. 410804-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Tebuthiuron.
Shaughnessey No: 105501.

2. **TEST MATERIAL:** Tebuthiuron (EL-103, Compound 75503); N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea; 99.08% active ingredient.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants, Tier 2. Species Tested: Anabaena flos-aquae.

4. **CITATION:** Negilski, D.S., D.W. Grothe, and P.J. Cocke 1989. Toxicity of Tebuthiuron to the blue-green alga (Anabaena flos-aquae) in a static test system. Prepared and submitted by Lilly Research Laboratories Division of Eli Lilly and Company, Greenfield, IN. MRID No. 410804-01.

5. **REVIEWED BY:**

Debra S. Segal, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Debra S. Segal
Date: 1-8-90
Charles R Lewis 5/23/90

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 1-10-90

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 5/25/90

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 growth and reproduction of a non-target green alga test. Based on percent inhibition of specific growth rate, the EC_{50} and EC_{25} were 15.1 and 2.99 mg/L, respectively. Based on cell count, the NOEC was 0.31 mg/L.
Day-5 EC_{50} and EC_{25} values were 4.06 and 1.69 mg/L, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

9.5 hrs.

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10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** Anabaena flos-aquae used in this test came from stock cultures maintained at the Environmental Toxicology Laboratory of Eli Lilly and Company. Originally, a sample of this species (UTEX No. 1444) was obtained from the Starr collection at the University of Texas. Stock cultures of A. flos-aquae were grown in algal nutrient medium and housed in an environmental growth chamber (Rheem-Shere, Model CEL 8).

B. **Dosage:** Seven-day growth and reproduction test.

C. **Test System:** Test vessels were 500-mL Erlenmeyer flasks made of borosilicate glass. Each flask contained 100 ml of solution. Temperature and pH of each treatment stock solution were measured at test initiation. At test termination these parameters were measured in each replicate test solution. Total alkalinity, total hardness, and conductivity of the aqueous nutrient medium were determined on day 0. Cultures were held at approximately 24 °C and continuously illuminated at 2 klux (40 uE/m^2/sec). The algal nutrient medium was prepared by adding 10.0 ml of each stock solution to 9.0 L of sterile water, and diluting to 10.0 L.

D. **Test Design:** Based on a seven-day study, six nominal concentrations of Tebuthiuron (0.31, 0.62, 1.25, 2.5, 5.0, and 10.0) were selected for the definitive test. Each treatment level consisted of three replicates.

An initial tebuthiuron main stock solution was made by adding 0.01006 g of test compound (corrected for purity) to 1000 ml of aqueous nutrient medium. This solution was mixed thoroughly with a mechanical stirrer. Individual stock solutions at each exposure level were made by adding the appropriate amounts of main stock solution and aqueous nutrient medium to a 500-ml Erlenmeyer flask.

A 1.0-ml sterilized pipette was used to transfer the appropriate volume of algal inoculum to each test flask in order to achieve a cell population density of 10,000 cells/ml. Each flask was capped with aluminum foil to prevent contamination while allowing free gas exchange, and placed in an environmental growth chamber for seven days. All flasks were agitated once a day to prevent the cells from clumping together. The location of each

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flask in the growth chamber was randomized on a daily basis to avoid possible light "hot spots".

Samples were collected for tebuthiuron analysis at test initiation from the treatment stock solutions that were used to fill each replicate flask. At test termination (day 7), samples were collected by filtering each test solution through a 0.7-um glass-fiber membrane filter to remove algal cells. Filtrates from treatment replicates were pooled and submitted for analysis of tebuthiuron.

The water samples were membrane filtered and diluted with methanol and mobile phase as appropriate to yield concentrations approximately that of analytical standards (0.4 ug/ml and 0.1 ug/ml) prepared in the same manner. Tebuthiuron was then assayed directly using high performance liquid chromatography. The chromatography was accomplished using a 4.6 mm X 250 mm Alltech C18-RP (10 um) column with 65:35 methanol:water mobile phase. The tebuthiuron was quantified by comparison to a tebuthiuron reference standard. Injections of 200 uL of 0.1 ug/ml (equivalent to 0.02 ug of tebuthiuron) permitted a limit of quantitation of approximately 0.012 mg/L for control water assayed in the same manner as the lowest level test sample.

Reproduction in the algal cultures was determined by quantifying cell populations on days 1, 2, 3, 4, 5, 6, and 7. A compound microscope and hemocytometer were used to enumerate the algal cells. Cell counts were expressed as number of algal cells per milliliter of solution (cells/ml). Each day it was necessary to sonicate a sample of the algal cells in order to break up clumps of cells. Approximately 1 ml of solution from a test vessel was placed in a glass liquid scintillation vial. The vial containing the solution was sonicated for approximately 20 minutes prior to counting on a hemocytometer. The limit of detection for this counting method was 104 cells/ml. To obtain a direct measure of algal biomass, dry weight of the algal cells in each flask was determined on day 7. A measured volume of solution from each flask was passed through a preweighed glass-fiber filter. Each filter was dried at 105 °C for 24 hours and reweighed. Dry weight of the algal cells was determined by calculation and expressed as milligrams of dry weight per milliliter of test solution (mg/ml).

E. **Statistics:** A one-tailed Dunnett's t-test was used to detect treatment responses that were significantly different ($p \leq 0.05$) from those of the control. To define the no-observed-effect concentration (NOEC), individual Dunnett's t-tests were performed on specific growth rates, on algal cell count data from day 7, and on the algal biomass data obtained from dry weight measurements on day 7. The specific growth rate of each replicate culture was determined as the slope of the growth curve (cell count versus time) during the logarithmic phase of algal reproduction (days 0 to 3) using the following regression equation:

$$\log_{10}(N) = Rt + \log_{10}(N_0), \text{ where}$$

N = cell count (cells/ml),
R = specific growth rate (1/day),
t = time (days), and
N_0 = initial cell count (10^4 cells/ml).

The median effective concentration was defined as the concentration of tebuthiuron that caused 50% inhibition of the specific growth rate of treated algal populations. The percent inhibition of specific growth rate at each tebuthiuron concentration was calculated with the following equation:

$$I_R = \frac{R_c - R_t}{R_c} \times 100, \text{ where}$$

I_R = percent inhibition based on specific growth rates,
R_c = mean of the specific growth rates of three replicate control cultures, and
R_t = mean of the specific growth rates of the three replicate cultures at each treatment level.

A linear regression of percent inhibition versus the logarithm of the average analyzed tebuthiuron concentration was used to obtain the median effective concentration. The 95% confidence interval around the regression line was generated using SAS, and a graph of the regression line and associated confidence limits was obtained. The 95% confidence limits for the median effective concentration (EC_{50}) were obtained by graphic interpolation.

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12. **REPORTED RESULTS:**

No significant decrease in specific growth rate relative to the control was observed at a mean tebuthiuron concentration of 0.31 mg/L (Table 3; attached). At mean tebuthiuron concentrations of ≥ 0.62 mg/L, specific growth rates were significantly lower than those in the control. Algal growth rates at tebuthiuron concentrations ≥ 0.62 mg/L ranged from 0.562 to 0.370 day $^{-1}$, compared to 0.634 day $^{-1}$ for the control. Based on specific growth rate, the NOEC for tebuthiuron was 0.31 mg/L.

Significant decreases in algal cell count and algal biomass occurred at tebuthiuron concentrations ≥0.62 mg/L (Table 4; attached). No significant reductions in algal cell counts or algal biomass occurred on test day 7 at a tebuthiuron concentration of 0.31 mg/L.

No significant reductions in algal biomass were observed at an average analyzed tebuthiuron concentration of 0.31 mg/L, where the mean dry weight value was 0.056 mg/ml compared to the control biomass of 0.04 mg/ml. Significant decreases in algal biomass occurred at tebuthiuron concentrations ≥0.62 mg/L. At tebuthiuron concentrations of 0.62, 1.32, 2.62, 5.49, and 11.05 mg/L, mean dry weight values were 0.036, 0.027, 0.018, 0.012, and 0.007 mg/ml, respectively. Based on algal cell count and biomass at test termination, the NOEC for tebuthiuron was 0.31 mg/L.

Using the logarithm of the average analyzed tebuthiuron concentration and the percent inhibition data for tebuthiuron concentrations ≥0.62 mg/L (Table 3), a linear regression model ($y = mx + b$) was used to estimate the median effective concentration. According to this analysis, the median effective concentration of tebuthiuron was estimated to be 30.9 mg/L with 95% confidence limits of 12.6 and 229 mg/L (Figure 2; attached). The slope of the regression line was 22.4, the y-intercept was 16.5, and the coefficient of determination (R^2) was 0.92.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

"Based on specific growth rate, algal cell count, and mean dry weight, the NOEC of tebuthiuron was 0.31 mg/L for the blue-green alga, Anabaena flos-aquae. These same parameters were significantly reduced relative to the control cultures at tebuthiuron concentrations ≥0.62

mg/L. Using the specific growth rate during the logarithmic phase of reproduction as an indicator of algal growth, the median effective concentration with 95% confidence limits was estimated to be 30.9 (12.6, 229) mg/L. The slope of the regression line was 22.4."

A GLP compliance statement was included in the report and the study was audited by Lilly research Laboratories' Quality Assurance Unit. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o The maximum label rate was not provided in the report.

o A 25% detrimental effect level was not provided in the study although it was calculated to be 2.99 mg/L.

o Aluminum foil was placed on the top of each flask to "prevent contamination while allowing free gas exchange". Although not stated in the SEP, aluminum foil probably did not allow for free gas exchange.

B. **Statistical Analysis:** The reviewer recalculated the EC50 value using linear regression by plotting the log of mean measured concentration against the percent inhibition of specific growth rate expressed as probits (attached) and obtained a value of 15.1 mg/L rather than 30.9 mg/L as reported by the authors. An EC_{25} value was calculated by the reviewer to be 2.99 mg/L. Dunnett's test was performed to compare cell counts and algal biomass at each treatment level to those of the solvent controls (attached). The results showed that concentrations of 0.62 mg/L reduced the cell counts of A. flos-aquae at test termination (day 7). The NOEC was calculated to be 0.31 mg/L.

C. **Discussion/Results:** The 7-day EC50 value of tebuthiuron (EL-103) was 15.1 mg/L based on % inhibition of specific growth rate. Based on the reduction of both cell counts and algal biomass, the no-observed-effect concentration

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(NOEC) was determined to be 0.31 mg/L nominal concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core

(2) **Rationale:** Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the validity of the toxicity results.

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** Yes, 01-05-90.

lewis tebuthiuron Anabaena 5-day

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
11.05	100	80	80	0
5.49	100	46	46	0
2.62	100	40	40	0
1.32	100	38	38	0
.62	100	0	0	0
.31	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 5.932558

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.709484E-02	[illegible]	[illegible]	3.235655

4.237821

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.4675192	10.22091	

0

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

[redacted] =
95 PERCENT CONFIDENCE LIMITS = .56343 AND 2.999806

EC 25 = 1.699
[redacted]
95 PERCENT CONFIDENCE LIMITS = 1.918451 AND 16.58471

[redacted]
95 PERCENT CONFIDENCE LIMITS = .0389392 AND 1.717353
****

lewis tebuthiuron anabaena 7-day
****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
11.05	100	88	88	0
5.49	100	68	68	0
2.62	100	55	55	0
1.32	100	52	52	0
.62	100	17	17	0
.31	100	0	0	0

THE BINOMIAL TEST SHOWS THAT .62 AND 1.32 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.268473

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	[redacted]	95 PERCENT CONFIDENCE LIMITS	
5	1.336607E-02		[redacted]	.920042

2.494296

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.236495	5.832722	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

[redacted]
95 PERCENT CONFIDENCE LIMITS = .8909471 AND 2.577848

EC 25 0.904
[redacted]
95 PERCENT CONFIDENCE LIMITS = 1.243045 AND 4.15792

[redacted]
95 PERCENT CONFIDENCE LIMITS = 7.650377E-02 AND .8157778

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****

slope = 1.699295 1.781618 LC50 = 4.064124 LC25 =

slope = .9040352 1.734398 LC50 = 2.213825 LC25 =

tebuthiuron anabaena 5-day
File: a:\anae Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	3603347.619	600557.937	85.736
Within (Error)	14	98066.667	7004.762	
Total	20	3701414.286		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

tebuthiuron anabaena 5-day
File: a:\anae Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	1410.000	1410.000		
2	.31	1461.667	1461.667	-0.756	
3	.62	1450.000	1450.000	-0.585	
4	1.32	868.333	868.333	7.926	*
5	2.62	840.000	840.000	8.341	*
6	5.49	766.667	766.667	9.414	*
7	11.05	278.333	278.333	16.560	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

tebuthiuron anabaena 5-day
File: a:\anae Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	3			
2	.31	3	172.891	12.3	-51.667
3	.62	3	172.891	12.3	-40.000
4	1.32	3	172.891	12.3	541.667
5	2.62	3	172.891	12.3	570.000
6	5.49	3	172.891	12.3	643.333
7	11.05	3	172.891	12.3	1131.667

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Tebuthiuron – Anabaena flos-aquae

EC50

$y = 3.876962 + 0.9521699x$

at $y = 5.0$:

$x = (5.0 - 3.876962)/0.9521699$

$x = 1.179$

inv. log = 15.1

EC50 = 15.1 mg/L

EC25 25% = 4.33 probits

$y = 4.33$

$x = (4.33 - 3.876962)/0.9521699$

$x = 0.4758$

inv. log = 2.99

EC25 = 2.99 mg/L

Anabaena

```
          3. DELETE SOME OF THE DATA
          4. PERFORM REGRESSION ANALYSIS
          5. STORE DATA
          6. GO TO PROGRAM MENU
          7. DO ANOTHER REGRESSION
OPTION ? 4

REGRESSION EQUATION:
Y= 3.876962 + .9521699 X

COEFFICIENT OF CORRELATION= .9300662


PRESS ENTER TO CONTINUE.?
          ACTUAL VERSUS ESTIMATED VALUES
          X=logconc  Y=probit
DATA POINT      X              Y              ESTIMATED Y    ERROR
 1             -.509           3.12            3.392307      -.2723072
 2             -.208           3.77            3.67891        9.108996E-02
 3              .121           4.36            3.992174       .367826
 4              .418           4.29            4.274969       1.503134E-02
 5              .74            4.45            4.581567      -.1315675
 6             1.043           4.8             4.870075      -7.007456E-02
PRESS ENTER TO CONTINUE?
```

Tebuthiuron - Anabaena flos-aquae

NOEC - cell count

Analysis of Variance File: tebana Date: 01-03-1989

FILTER: None

N's, means and standard deviations based on dependent variable: COUNT

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean - cell count	S.D.
*		21	1399.5238	771.0705
1	Control	3	2325.0000	107.5872
2	.31 mg/L	3	2360.0000	47.6970
3	0.62	3	1926.6666	43.1084
4	1.32	3	1118.3334	28.8675
5	2.62	3	1051.6666	16.0728
6	5.49	3	733.3333	53.4634
7	11.05	3	281.6667	16.0728

Fmax for testing homogeneity of between subjects variances: 44.81
Number of variances= 7 df per variance= 2.

Analysis of Variance Dependent variable: COUNT

Source	df	SS (H)	MSS	F	P
Between Subjects	20	11890994.0000			
C (CONC)	6	11851162.0000	1975193.6200	694.234	0.0000
Subj w Groups	14	39832.0000	2845.1428		

NOEC - cell count

Analysis of Variance File: tebana Date: 01-03-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	2325.000	6	733.333
2	2360.000	7	281.667
3	1926.667		
4	1118.333		
5	1051.667		

Comparison	Tukey-A*	Dunnett
1 < 2		
1 > 3	0.0100	0.0100
1 > 4	0.0100	0.0100
1 > 5	0.0100	0.0100
1 > 6	0.0100	0.0100
1 > 7	0.0100	0.0100
2 > 3	0.0100	N.A.
2 > 4	0.0100	N.A.
2 > 5	0.0100	N.A.
2 > 6	0.0100	N.A.
2 > 7	0.0100	N.A.
3 > 4	0.0100	N.A.
3 > 5	0.0100	N.A.
3 > 6	0.0100	N.A.
3 > 7	0.0100	N.A.
4 > 5		N.A.
4 > 6	0.0100	N.A.
4 > 7	0.0100	N.A.
5 > 6	0.0100	N.A.
5 > 7	0.0100	N.A.
6 > 7	0.0100	N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500). A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

NOEC- biomass

Analysis of Variance File: tebana Date: 01-03-1989

FILTER: None

N's, means and standard deviations based on dependent variable: INHIB

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean - biomass	S.D.
	*	21	0.0278	0.0162
	1 - Control	3	0.0397	0.0025
	2 0.3 mg/L	3	0.0553	0.0012
	3 0.62	3	0.0357	0.0006
	4 1.32	3	0.0267	0.0015
	5 2.62	3	0.0177	0.0025
	6 5.49	3	0.0123	0.0021
	7 11.05	3	0.0070	0.0010

Fmax for testing homogeneity of between subjects variances: 19.00
Number of variances= 7 df per variance= 2.

Analysis of Variance Dependent variable: INHIB

Source	df	SS (H)	MSS	F	P
Between Subjects	20	0.0053			
C (CONC)	6	0.0052	0.0009	276.281	0.0000
Subj w Groups	14	0.0000	0.0000		

NOEC- biomass

Analysis of Variance File: tebana Date: 01-03-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	0.040	6	0.012
2	0.055	7	0.007
3	0.036		
4	0.027		
5	0.018		

Comparison	Tukey-A*	Dunnett
1 < 2	0.0100	0.0100
1 > 3		
1 > 4	0.0100	0.0100
1 > 5	0.0100	0.0100
1 > 6	0.0100	0.0100
1 > 7	0.0100	0.0100
2 > 3	0.0100	N.A.
2 > 4	0.0100	N.A.
2 > 5	0.0100	N.A.
2 > 6	0.0100	N.A.
2 > 7	0.0100	N.A.
3 > 4	0.0100	N.A.
3 > 5	0.0100	N.A.
3 > 6	0.0100	N.A.
3 > 7	0.0100	N.A.
4 > 5	0.0100	N.A.
4 > 6	0.0100	N.A.
4 > 7	0.0100	N.A.
5 > 6	0.0500	N.A.
5 > 7	0.0100	N.A.
6 > 7	0.0500	N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500). A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

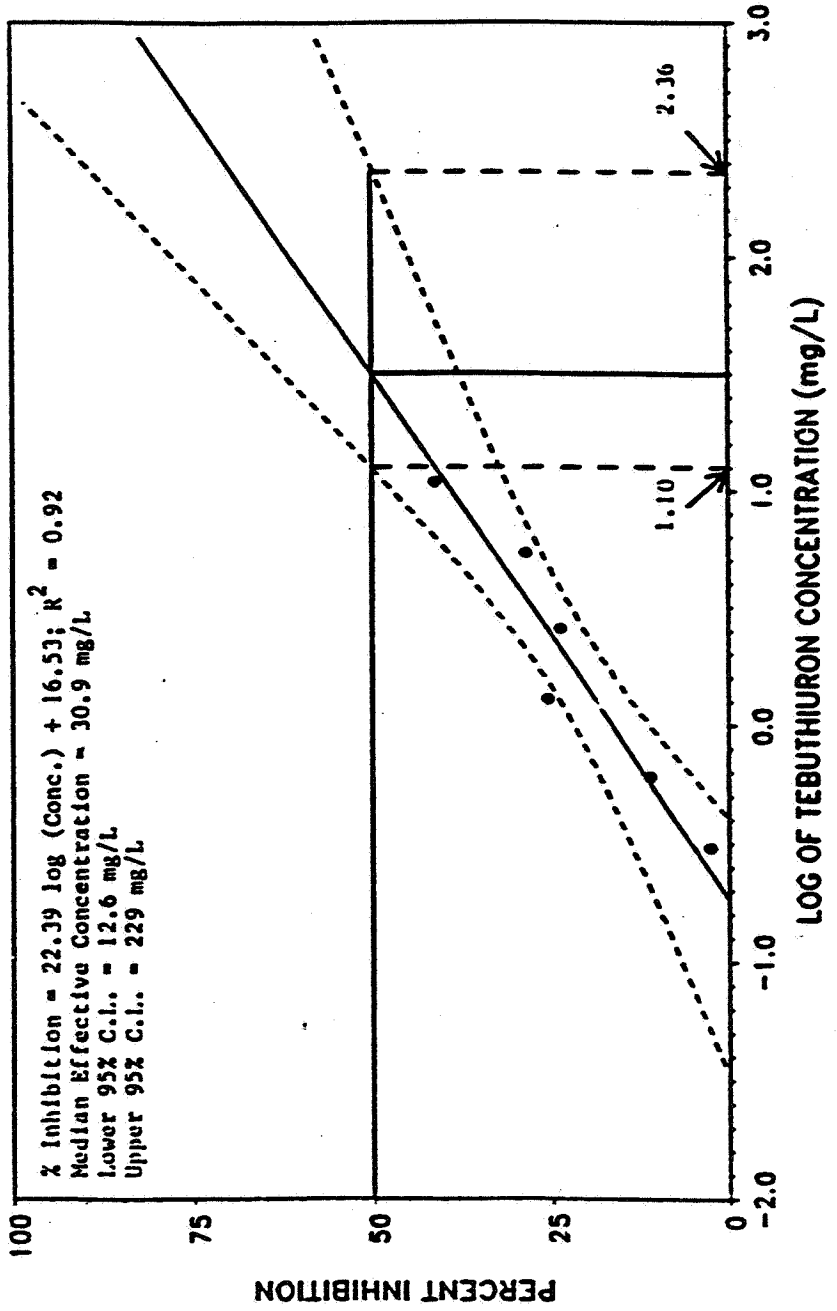

FIGURE 2. REGRESSION CURVE USED IN ESTIMATING THE 95% CONFIDENCE LIMITS FOR THE MEDIAN EFFECTIVE CONCENTRATION OF TEBUTHIURON FOR Anabaena flos-aquae, STUDY J00489

TABLE 3. INHIBITION OF Anabaena flos-aquae REPRODUCTION BY TEBUTHIURON DURING THE LOGARITHMIC GROWTH PHASE (DAYS 0 TO 3) AS MEASURED BY SPECIFIC GROWTH RATE. STUDY J00489.

Average Analyzed Tebuthiuron Concentration (mg/L)	Specific Growth Rate[a] (day^{-1})	Percent Inhibition[b]
ND (Control)	0.634 ±0.020	0.0
0.31	0.615 ±0.005	3.0
0.62	0.562* ±0.018	11.4
1.32	0.469* ±0.016	26.0
2.62	0.480* ±0.014	24.3
5.49	0.451* ±0.029	28.9
11.05	0.370* ±0.012	41.6

* Significantly reduced compared to the control value ($p \leq 0.05$).

[a] Mean ± SD, n = 3. The growth rate of each replicate culture was estimated with the regression equation: $\log_{10}(N) = R \cdot t + \log_{10}(N_0)$, where N = cell count (cells/ml),
R = specific growth rate (1/day),
t = time (days), and
N_0 = initial cell count (10^4 cells/ml).

[b] Calculated by the equation: $I_R = \frac{R_c - R_t}{R_c} \times 100$,

where I_R = percent inhibition based on average specific growth rate,
R_c = mean of the specific growth rates of three replicate control cultures, and
R_t = mean of the specific growth rates of the three replicate cultures at each treatment level.

TABLE 4. ALGAL CELL COUNTS AND BIOMASS OF Anabaena flos-aquae POPULATIONS EXPOSED TO TEBUTHIURON FOR SEVEN DAYS. STUDY J00489.

Average Analyzed Tebuthiuron Concentration (mg/L)	Cell Counts On Day 7 (10^3 cells/ml)	Algal Biomass On Day 7 (mg/ml)
ND (Control)	2325 ± 108	0.040 ±0.003
0.31	2360 ± 48	0.056 ±0.001
0.62	1927* ± 43	0.036* ±0.010
1.32	1118* ± 29	0.027* ±0.002
2.62	1052* ± 16	0.018* ±0.003
5.49	733* ± 54	0.012* ±0.002
11.05	282* ± 16	0.007* ±0.001

[a] Mean ± SD, n=3. Measured as dry weight of algal cells.

* Significantly reduced compared to the water control ($p \leq 0.05$).

Shaughnessey No. 105501 Chemical Name [illegible] Chemical Class ____ Page ____ of ____

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Valid Stu
14-Day Single Dose Oral LD_{50}		LD50 = . mg/kg (95% C.L.) Contr. Mort.(%)=		
Species ____		Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species ____		Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =		
Lab ____		48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
~~96-Hour LC_{50}~~ 7-Day EC_{50}		EC50 = 15.1 ppm (95% C.L.) Con. Mor.(%)= N/A		
Species Anabaena flos-aquae 99.08%		Slope= 22.4 # Animals/Level= cells/ml 10,000 Sol. Con. Mor.(%)= N/A	D.S. 1-5-90	Core
Lab Eli Lilly		~~96-Hour~~ 7-day Dose Level ppm/(%~~Mortality~~ % inhibition) Temp.= 24°C		
Acc. 410804-01		0.31 (3.0), 0.62 (11.4), 1.32 (26.0), 2.62 (24.3), 5.49 (28.9), 11.05 (41.6)		
		Comments: Based on mean measured concentrations.		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ____		96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		

File

MRID No. 410804-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Tebuthiuron.
Shaughnessey No. 105501.

2. **TEST MATERIAL:** Technical tebuthiuron (EL-103, compound 75503); chemical name: N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea; Lot No. 729-AS7; 99.08% active ingredient.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Skeletonema costatum.

4. **CITATION:** Negilski, D.S. and P.J. Cocke. 1989. Toxicity of Tebuthiuron to a Marine Diatom (Skeletonema costatum) in a Static Test System. Laboratory Project No. J00389. Conducted by Lilly Research Laboratories, Greenfield, Indiana. Submitted by Elanco Products Company, MRID No. 410804-02.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: January 10, 1990
Charles A. Lum 5/23/90

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 1-10-90

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven 5/20/90
Date:

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier-2 growth and reproduction test using a marine diatom. Based on the cell counts on day 7, the EC25 and EC50 values of tebuthiuron for Skeletonema costatum were 0.036 and 0.067 mg/L mean measured concentrations, respectively. Based on the cell counts and biomass on day 7 and the calculated EC25, the NOEC value was determined to be <0.036 mg/L mean measured concentration. Day-5 EC50 and EC25 values were 0.05 and 0.031 mg/L, respectively.

8. **RECOMMENDATIONS:** N/A.

9 hrs.

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9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** The marine diatom, Skeletonema costatum, used in this test came from stock cultures maintained at the testing facility. The original culture was obtained from the Starr collection at the University of Texas. Stock cultures were held in an environmental chamber at a temperature of approximately 20°C under a photoperiod of 16 hours of light and 8 hours of darkness. The light intensity was approximately 4 klux.

The marine algal nutrient medium used in maintaining stock cultures and testing was the Aquil medium described by Morel et al., (1979). Procedures used to prepare the nutrient medium were taken from Walsh (1988). The pH of the medium was adjusted to approximately 8.0 using NaOH or HCl.

B. **Dosage:** Seven-day growth and reproduction test.

C. **Test System and Design:** Test vessels were 500-ml Erlenmeyer flasks, each containing 100 ml of an appropriate test solution. Based on a pilot study, seven nominal concentrations of tebuthiuron (0.002, 0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 mg/L) and a control were employed in the definitive test. Three replicates were included at each treatment level and the control.

The test was initiated when an inoculum was added to each flask, yielding a cell population density of 10,000 cells/ml. Each flask was capped with aluminum foil to prevent contamination while allowing free gas exchange. The flasks were placed in an environmental growth chamber. The temperature, photoperiod, and light intensity employed during the test were the same as those used for culturing. The flasks were agitated once a day to minimize clumping of cells. The location of each flask in the growth chamber was randomized daily.

A compound microscope and hemocytometer were used to perform cell counts (cells/ml) on days 1, 2, 3, 4, 5,

and 7. The dry weight of diatom cells in each flask was determined on day 7 as a direct measurement of the biomass (mg dry weight/ml of test solution).

Test solution samples were collected at test initiation and termination for tebuthiuron analysis, using high performance liquid chromatography (HPLC). The samples from treatment replicates at test termination were filtered and pooled before the analysis. The temperature and pH of each test solution were measured at test initiation. At test termination, these parameters were measured in each replicate test solution. Total alkalinity, total hardness, and conductivity of the aqueous nutrient medium were determined on day 0.

E. **Statistics:** To determine the no-observed-effect concentration (NOEC), treatment responses (i.e., specific growth rates, cell counts on day 7, and biomass on day 7) were compared to the control responses using a one-tailed Dunnett's t-test. The specific growth rate of each replicate culture was determined as the slope of the growth curve during the logarithmic phase using the following equation:

$$\log (N) = (R \times t) + \log (N_0)$$

where: N = cell count (cells/ml),
R = specific growth rate (day^{-1}),
t = time (days), and
N_0 = initial cell count (10,000 cells/ml).

The percent inhibition of specific growth rate at each tebuthiuron concentration was calculated using the following equation:

$$I_R = \frac{R_c - R_t}{R_c} \times 100$$

where: I_R = percent inhibition based on specific growth rate,
R_c = mean of the specific growth rates of the three-replicate control cultures, and
R_t = mean of the specific growth rates of the three-replicate cultures at each treatment level.

The median effective concentration (EC50) and its corresponding 95% confidence limits were determined by a linear regression of percent inhibition versus the

logarithm of mean measured concentrations using SAS program.

12. **REPORTED RESULTS:** During the test, the temperature remained between 18.5 and 21.3°C in all solutions. However, the mean temperature of all test solutions temporarily increased to 24.8°C at test initiation but was promptly adjusted to 20.5°C within 3.5 hours. The pH of treatment solutions ranged from 7.9 to 8.1 and 8.4 to 8.7 at test initiation and termination, respectively. The total hardness, total alkalinity, and conductivity of the nutrient medium at test initiation were >2500 mg/L as $CaCO_3$, 120 mg/L as $CaCO_3$, and 23.4 mS/cm, respectively.

The tebuthiuron concentration at each treatment remained relatively stable over the 7-day test period. The mean measured concentrations were 0.0018, 0.0092, 0.018, 0.038, 0.076, 0.16, and 0.30 mg/L, representing 90 to 100% of the nominal values.

Table 3 (attached) presents specific growth rates for the control and each treatment level. The cell number increased from 10,000 cells/ml to a mean of 195,000 cells/ml during the first three days of the test. Therefore, this period was considered a logarithmic phase. After day 3, growth rates of the control cultures decreased, indicating a transition into a stationary phase. No significant decrease in specific growth rate relative to the control was observed at mean measured concentrations of $\leq$0.038 mg/L. Specific growth rates at test concentrations of $\geq$0.076 mg/L were significantly ($p \leq 0.05$) lower than those of the control. Based on the mean specific growth rate, the NOEC for tebuthiuron was 0.038 mg/L.

Mean cell counts and biomass of the diatom at test termination (day 7) are presented in Table 4 (attached). At mean measured concentrations of $\leq$0.038 mg/L, the cell counts on day 7 were not significantly reduced when compared to those in the control. There was a significant decrease in biomass at test concentration of 0.0092 mg/L when compared to the control. However, the authors suggested that the decrease was not dose-related since the biomass at two higher concentrations (0.018 and 0.038 mg/L) were higher than those in the control. Significant decreases in cell counts and biomass were found between the control values and those at concentrations of $\geq$0.76 mg/L. Based on the cell counts and biomass at test termination, the NOEC for tebuthiuron was 0.038 mg/L.

The authors stated that the biomass data might not be a

2c

reliable measure of standing crop for S. costatum. At the three highest concentrations (0.076, 0.16, 0.30 mg/L), the mean biomass values were between 69 and 74% of the control value, while the mean cell counts at those levels were between <4 and 27% of the control value. They suggested that this discrepancy was probably due to the precipitation of salts from the marine nutrient medium onto the filter disks used for the determinations of the diatom dry weights.

The calculated values of growth inhibition for the control and each treatment were shown in Table 3 (attached). The percentage inhibition data at test concentrations of ≥0.018 were used to calculate the EC50 value. The EC50 determined from a regression analysis was 0.101 mg/L with a 95% confidence interval of 0.068 and 0.174 mg/L. The slope of the regression line and the y-intercept were 59.39 and 109.09, respectively. The coefficient of determination (R^2) was 0.95.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The NOEC of tebuthiuron for S. costatum was 0.038 mg/L. When compared to the control cultures, specific growth rates, cell counts, and biomass were significantly reduced at test concentrations of ≥0.076 mg/L. Using the specific growth rate during the logarithmic phase of reproduction as an indicator of the diatom growth, the EC50 value with 95% confidence limits was 0.101 (0.068-0.174) mg/L. The slope of the dose-response curve was 59.39.

Several inspections had been conducted during the course of the study by the Quality Assurance Unit of Lilly Research Laboratories for compliance with the OECD GLP standards. A GLP statement was included in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o The maximum label rate was not provided in the report. Therefore, it could not be determined if the concentrations tested were less than the maximum label rate as though it were applied directly to the surface of a 15-cm water column.

o The composition of the nutrient medium used in culturing and testing should have been described in the report.

o The EC25 value was not reported.

It was reported that each flask was capped with aluminum foil to prevent contamination while allowing free gas exchange. Foam or a wrapped cotton ball is probably a better material for this purpose.

B. **Statistical Analysis:** The reviewer calculated EC50 and EC25 values for each growth parameter using a regression analysis. All calculations are attached. The EC50 value based on specific growth rate (0.102 mg/L) was similar to that calculated by the author (0.101 mg/L). However, the estimation based on the cell counts yielded the lowest EC50 and EC25 (i.e., 0.067 and 0.036 mg/L, respectively). Therefore, these values should be used in the risk assessment of tebuthiuron.

Analysis of variance (ANOVA) with a multiple comparison (Dunnett's) test was performed on the day-7 cell count and day-7 biomass to compare the values at each treatment level to those of the control. The printouts are attached. The results showed that test concentrations of $\geq$0.076 mg/L significantly ($p = 0.01$) decreased the cell counts and biomass of S. costatum when compared to the control values. This is the same as those analyzed by the author, except the reviewer did not find a significant decrease in biomass at 0.0092 mg/L. Since the raw data on specific growth rate were not submitted, the ANOVA on this parameter could not be verified.

C. **Discussion/Results:** This study is scientifically sound. Based on the cell counts on day 7, the EC25 and EC50 values of tebuthiuron for S. costatum were 0.036 and 0.067 mg/L mean measured concentrations, respectively. Based on the decrease in cell counts and biomass at tebuthiuron test concentrations of $\geq$0.076 mg/L and the calculated EC25 above, the NOEC was determined to be <0.036 mg/L.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, January 9, 1990.

TABLE 3. INHIBITION OF Skeletonema costatum REPRODUCTION BY TEBUTHIURON DURING THE LOGARITHMIC GROWTH PHASE (DAYS 0 TO 3) AS MEASURED BY SPECIFIC GROWTH RATE. STUDY J00389.

Log Conc	Average Analyzed Tebuthiuron Concentration (mg/L)	Specific Growth Rate[a] (1/day)	Percent Inhibition[b]
	ND (Control)	0.449 ±0.026	0.0
−2.7447	0.0018	0.435 ±0.060	3.2
−2.0362	0.0092	0.474 ±0.036	-5.6
−1.7447	0.018	0.426 ±0.049	5.2
−1.4202	0.038	0.337 ±0.052	25.0
−1.1192	0.076	0.278* ±0.064	38.1
−0.7959	0.16	0.125* ±0.101	72.2
−0.5229	0.30	0.125* ±0.160	72.3

* Significantly less than the control ($p \leq 0.05$).

[a] Mean ± SD, n=3. The growth rate of each replicate culture was estimated with the regression equation: $\log_{10}(N) = R \cdot t + \log_{10}(N_0)$, where N = cell count (cells/ml),
R = specific growth rate (day^{-1}),
t = time (days), and
N_0 = initial cell count (10^4 cells/ml).

[b] Calculated by the equation: $I_R = \frac{R_c - R_t}{R_c} \times 100$,
where I_R = percent inhibition based on specific growth rate,
R_c = mean of the specific growth rates of the three replicate control cultures, and
R_t = mean of the specific growth rates of the three replicate cultures at each treatment level.

TABLE 4. CELL COUNTS AND BIOMASS OF Skeletonema costatum POPULATIONS EXPOSED TO TEBUTHIURON FOR SEVEN DAYS. STUDY J00389.

Log conc.	Average Analyzed Tebuthiuron Concentration (mg/L)	Cell Count On Day 7 (10^3 cells/ml)	% I	Diatom Biomass[a] On Day 7 (mg/ml)	% I
	ND (Control)	257 ±15		0.154 ±0.029	
−2.7447	0.0018	345 ±5	−34	0.133 ±0.013	14
−2.0362	0.0092	280 ±50	−9	0.123* ±0.008	20
−1.7447	0.018	308 ±38	−20	0.140 ±0.008	9
−1.4202	0.038	225 ±10	12	0.127 ±0.006	18
−1.1192	0.076	70* ±13	73	0.106* ±0.006	31
−0.7959	0.16	12* ±13	95	0.114* ±0.012	26
−0.5229	0.30	<10* ±0	>96	0.110* ±0.003	29

* Significantly less than the control ($p \leq 0.05$).

[a] Mean ± SD, n=3. Measured as dry weight of diatom cells.

Technical

Sh. No. 105501 Chemical Name Tebuthiuron Chemical Class ___ Page 1 of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validati Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
Species ___		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L) Contr. Mort.(%)=		
Species ___		Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ___		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab ___		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =		
Lab ___		48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
~~96-Hour~~ 7-Day EC_{50}		EC50 = 0.067 ppm* (95% C.L.) Based on cell counts Con. Mor(%)= N/A		
Species Skeletonema costatum		Slope= 91.56** # Animals/Level= cells/ml = 10,000 Sol. Con. Mor.(%)=N/A	PK 1-9-90	Core
Lab Lilly Research Laboratories 99.08		~~96-Hour~~ 7-Day Dose Level ppm/(%Mortality) Inhibition Temp.= 20C 0.038(12), 0.074(31), 0.16(95), 0.30(96), ( )		
Acc. MRID 41080402		Comments: * mean measured concentrations, ** X = log concentration Y = % inhibition a = 157.31		
96-Hour LC_{50}		LC50 = pp__ (95% C.L) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab ___		96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		

3, 9

Skeletonema costatum

Specific Growth rate

REGRESSION EQUATION:
Y= 108.8435 + 59.29387 X

COEFFICIENT OF CORRELATION= .9764648

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=PERCENT INHIBITION (GROWTH RATE)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	-1.7447	5	5.393509	-.3935089
2	-1.4202	25	24.63437	.3656311
3	-1.1192	38	42.48183	-4.481827
4	-.7959	72	61.65154	10.34847
5	-.5229	72	77.83876	-5.83876

EC_{25} = 0.039 mg/L
EC_{50} = 0.102 "

Cell counts

REGRESSION EQUATION:
Y= 157.3149 + 91.56076 X

COEFFICIENT OF CORRELATION= .9037976

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION] Y=PERCENT INHIBITION (CELL COUNTS)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	-1.4202	12	27.28035	-15.28035
2	-1.1192	73	54.84014	18.15986
3	-.7959	95	84.44173	10.55827
4	-.5229	96	109.4378	-13.43782

EC_{25} = 0.036 mg/L
EC_{50} = 0.067 "

S. costatum

Biomass

REGRESSION EQUATION:
Y= 32.49329 + 7.747934 X

COEFFICIENT OF CORRELATION= .7317549

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=PERCENT INHIBITION (BIOMASS)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	-2.7447	14	11.22753	2.772467
2	-2.0362	20	16.71694	3.283058
3	-1.7447	9	18.97547	-9.975468
4	-1.4202	18	21.48967	-3.48967
5	-1.1192	31	23.8218	7.178202
6	-.7959	26	26.32671	-.3267059
7	-.5229	29	28.44189	.5581093

EC_{25} = 0.108 mg/L

EC_{50} = 181.77 " (Extrapolated)

Analysis of Variance File: TEBU1 Date: 01-08-1989

FILTER: None

N's, means and standard deviations based on dependent variable: COUNTS

* Indicates statistics are collapsed over this factor

Factors: C			N	Mean	S.D.
*			24	188750.0000	131341.3280
1	Control		3	256666.6720	15275.2520
2	0.0018	mg/L	3	345000.0000	5000.0000
3	0.0092	"	3	280000.0000	50000.0000
4	0.018	"	3	308333.3400	38188.1290
5	0.038	"	3	225000.0000	10000.0000
6	0.076	"	3	70000.0000	13228.7568
7	0.16	"	3	15000.0000	8660.2539
8	0.30	"	3	10000.0000	0.0000

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

Fmax for testing homogeneity of between subjects variances: Not defined

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

Analysis of Variance Dependent variable: COUNTS

```
Source               df         SS (H)          MSS           F        P
Between Subjects    23%396762480000.0000
  C (CONC)           7%387629150000.0000%55375593000.0000   97.008   0.0000
  Subj w Groups     16%9133326300.0000%570832900.0000
```

12

Analysis of Variance File: TEBU1 Date: 01-08-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	256666.672	6	70000.000
2	345000.000	7	15000.000
3	280000.000	8	10000.000
4	308333.340		
5	225000.000		

Comparison	Dunnett	
1 < 2	0.0100	
1 < 3		
1 < 4		
1 > 5		
1 > 6	0.0100 *	0.076 mg/L
1 > 7	0.0100 *	0.16 "
1 > 8	0.0100 *	0.30 "
2 > 3	N.A.	
2 > 4	N.A.	
2 > 5	N.A.	
2 > 6	N.A.	
2 > 7	N.A.	
2 > 8	N.A.	
3 < 4	N.A.	
3 > 5	N.A.	
3 > 6	N.A.	
3 > 7	N.A.	
3 > 8	N.A.	
4 > 5	N.A.	
4 > 6	N.A.	
4 > 7	N.A.	
4 > 8	N.A.	
5 > 6	N.A.	
5 > 7	N.A.	
5 > 8	N.A.	
6 > 7	N.A.	
6 > 8	N.A.	
7 > 8	N.A.	

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Analysis of Variance File: TEBUT2 Date: 01-08-1989

FILTER: None

N's, means and standard deviations based on dependent variable: BIOMASS

* Indicates statistics are collapsed over this factor

Factors: C			N	Mean	S.D.
*			24	0.1260	0.0188
1	Control		3	0.1537	0.0287
2	0.0018	mg/L	3	0.1330	0.0132
3	0.0092	"	3	0.1233	0.0078
4	0.018	"	3	0.1407	0.0085
5	0.038	"	3	0.1273	0.0061
6	0.076	"	3	0.1063	0.0061
7	0.16	"	3	0.1137	0.0116
8	0.30	"	3	0.1103	0.0031

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

Fmax for testing homogeneity of between subjects variances: 88.43
Number of variances= 8 df per variance= 2.

DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD

Analysis of Variance Dependent variable: BIOMASS

Source	df	SS (H)	MSS	F	P
Between Subjects	23	0.0082			
C (CONC)	7	0.0055	0.0008	4.625	0.0053
Subj w Groups	16	0.0027	0.0002		

Analysis of Variance File: TEBUT2 Date: 01-08-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	0.154	6	0.106
2	0.133	7	0.114
3	0.123	8	0.110
4	0.141		
5	0.127		

Comparison	Dunnett		
1 > 2			
1 > 3			
1 > 4			
1 > 5			
1 > 6	0.0100	*	0.076 mg/L
1 > 7	0.0100	*	0.16 "
1 > 8	0.0100	*	0.30 "
2 > 3	N.A.		
2 < 4	N.A.		
2 > 5	N.A.		
2 > 6	N.A.		
2 > 7	N.A.		
2 > 8	N.A.		
3 < 4	N.A.		
3 < 5	N.A.		
3 > 6	N.A.		
3 > 7	N.A.		
3 > 8	N.A.		
4 > 5	N.A.		
4 > 6	N.A.		
4 > 7	N.A.		
4 > 8	N.A.		
5 > 6	N.A.		
5 > 7	N.A.		
5 > 8	N.A.		
6 < 7	N.A.		
6 < 8	N.A.		
7 > 8	N.A.		

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

tebuthiuron skeletonema 5-day
File: a:\skel Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	300082.292	42868.899	43.184
Within (Error)	16	15883.333	992.708	
Total	23	315965.625		

Critical F value = 2.66 (0.05,7,16)
Since F > Critical F REJECT Ho:All groups equal

tebuthiuron skeletonema 5-day
File: a:\skel Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	210.000	210.000		
2	.0018	291.667	291.667	-3.175	
3	.0092	186.667	186.667	0.907	
4	.018	276.667	276.667	-2.591	
5	.038	191.667	191.667	0.713	
6	.076	35.000	35.000	6.803	*
7	.16	3.333	3.333	8.034	*
8	.3	0.000	0.000	8.163	*

Dunnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

tebuthiuron skeletonema 5-day
File: a:\skel Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	3			
2	.0018	3	65.857	31.4	-81.667
3	.0092	3	65.857	31.4	23.333
4	.018	3	65.857	31.4	-66.667
5	.038	3	65.857	31.4	18.333
6	.076	3	65.857	31.4	175.000
7	.16	3	65.857	31.4	206.667
8	.3	3	65.857	31.4	210.000

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.259342
95 PERCENT CONFIDENCE LIMITS =-.1276934 AND 6.646378

LC50 = 5.024897E-02
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 2.048699E-02
95 PERCENT CONFIDENCE LIMITS = 0 AND 4.613237E-02

****

lewis tebuthiuron skeltonema 7-day

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.3	100	100	100	0
.16	100	95	95	0
.076	100	73	73	0
.038	100	13	13	0
.018	100	0	0	0
.0092	100	0	0	0
.0018	100	0	0	0

THE BINOMIAL TEST SHOWS THAT .038 AND .076 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 5.900741E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	6.924388E-03	.0594295	5.258654E-02	.0675646

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	2.675621E-02	1	.3224515

SLOPE = 4.83398
95 PERCENT CONFIDENCE LIMITS = 4.04327 AND 5.62469

LC50 = 6.228613E-02 EC 25 = 0.0451934?
95 PERCENT CONFIDENCE LIMITS = 5.714577E-02 AND 6.790111E-02

LC10 = .0340148
95 PERCENT CONFIDENCE LIMITS = 2.929347E-02 AND 3.821183E-02

****

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lewis tebuthiuron skeletonema 5-day

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.3	100	100	100	0
.16	100	99	99	0
.076	100	83	83	0
.038	100	9	9	0
.018	100	0	0	0
.0092	100	11	11	0
.0018	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 5.644687E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	7.235287E-03		[illegible]7213E-02	
4.173417E-02		5.347176E-02		

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	1.07989	35.11741	

0.05

slope = .0312189 3.259342 LC50 = 5.024897E-02 LC25 = 0.0312189

slope = 4.519347E-02 4.83398 LC50 = 6.228613E-02 LC25 = .0451934

File

MRID No. 410804-03

DATA EVALUATION RECORD

1. **CHEMICAL:** Tebuthiuron.
Shaughnessey No: 105501.

2. **TEST MATERIAL:** Tebuthiuron (EL-103, Compound 75503); N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea; 99.08% active ingredient.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants, Tier 2. Species Tested: Navicula pelliculosa.

4. **CITATION:** Negilski, D.S. and P.J. Cocke 1989. Toxicity of Tebuthiuron to a freshwater diatom (Navicula pelliculosa) in a static test system. Prepared and submitted by Lilly Research Laboratories Division of Eli Lilly and Company, Greenfield, IN. MRID No. 410804-03.

5. **REVIEWED BY:**

Debra S. Segal, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Debra S Segal
Date: 1-8-90
Charles Lewis 5/23/90

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 1-10-90

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven 5/25/90
Date:

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 growth and reproduction of a non-target diatom test. Based on percent inhibition of specific growth rate, the EC_{50} and EC_{25} were 0.193 and 0.111 mg/L, respectively. Based on diatom biomass, the NOEC was 0.056 mg/L.
Day 5 EC50 and EC25 values were 0.081 and 0.035 mg/L, respectively.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

9.5 hrs.

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10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** Navicula pelliculosa used in this test came from stock cultures maintained at the Environmental Toxicology Laboratory of Eli Lilly and Company. Originally, a sample of this species (UTEX No. 644) was obtained from the Starr collection at the University of Texas. Stock cultures of Navicula pelliculosa were grown in algal nutrient medium and housed in an environmental growth chamber (Rheem-Shere, Model CEL 8).

B. **Dosage:** Seven-day growth and reproduction test.

C. **Test System:** Test vessels were 500-mL Erlenmeyer flasks made of borosilicate glass. Each flask contained 100 ml of solution. Exposure solutions were held at about 24 °C and continuously illuminated by a combination of wide spectrum fluorescent, cool white fluorescent, and 100 W incandescent bulbs at an intensity of approximately 4.3 klux at the surface of the solutions. Cultures were held at approximately 24 °C and continuously illuminated at 4.3 klux (85 uE/m^2/sec). The algal nutrient medium was prepared by adding 10.0 ml of each stock solution to 9.0 L of sterile water, and diluting to 10.0 L.

D. **Test Design:** Based on a seven-day study, seven nominal concentrations of Tebuthiuron (0.0012, 0.011, 0.056, 0.11, 0.22, 0.46, and 0.89 mg/L) were selected for the definitive test. Each treatment level consisted of three replicates.

An initial tebuthiuron main stock solution was made by adding 0.01006 g of test compound (corrected for purity) to 1000 ml of aqueous nutrient medium. This solution was mixed thoroughly with a mechanical stirrer. Individual stock solutions at each exposure level were made by adding the appropriate amounts of main stock solution and aqueous nutrient medium to a 500-ml Erlenmeyer flask.

A 1.0-ml sterilized pipette was used to transfer the appropriate volume of diatom inoculum to each test flask in order to achieve a cell population density of 10,000 cells/ml. Each flask was capped with aluminum foil to prevent contamination while allowing free gas exchange, and placed in an environmental growth chamber for seven days. All flasks were agitated once a day to prevent

the cells from clumping together. The location of each flask in the growth chamber was randomized on a daily basis to avoid possible light "hot spots".

Samples were collected for tebuthiuron analysis at test initiation from the treatment stock solutions that were used to fill each replicate flask. At test termination (day 7), samples were collected by filtering each test solution through a 0.7-um glass-fiber membrane filter to remove algal cells. Filtrates from treatment replicates were pooled and submitted for analysis of tebuthiuron.

The tebuthiuron was extracted from the test solutions by liquid:liquid partition using dichloromethane. The dichloromethane was removed from the extracts and the residues redissolved in appropriate volumes of mobile phase. Tebuthiuron was measured using high performance liquid chromatography.

Reproduction in the diatom cultures was determined by quantifying cell populations on days 1, 2, 3, 4, 5, and 7. A compound microscope and hemocytometer were used to enumerate the diatom cells. Cell counts were expressed as number of diatom cells per milliliter of solution (cells/ml). Prior to making cell counts, diatom cells were removed from the sides and bottom of each test vessel. This was accomplished by rubbing the sides and bottom of each vessel with a piece of split tygon tubing that was slowly rotating on the end of a mechanical mixer shaft. To obtain a direct measure of diatom biomass, dry weight of the diatom cells in each flask was determined on day 7. A measured volume of solution from each flask was passed through a preweighed glass-fiber filter. Each filter was dried at 105 °C for 24 hours and reweighed. Dry weight of the diatom cells was determined by calculation and expressed as milligrams of dry weight per milliliter of test solution (mg/ml).

E. **Statistics:** A one-tailed Dunnett's t-test was used to detect treatment responses that were significantly different ($p \leq 0.05$) from those of the control. To define the no-observed-effect concentration (NOEC), individual Dunnett's t-tests were performed on specific growth rates, on diatom cell count data from day 7, and on the diatom biomass data obtained from dry weight measurements on day 7. The specific growth rate of each replicate culture was determined as the slope of the growth curve (cell count versus time) during the

logarithmic phase of algal reproduction (days 1 to 7) using the following regression equation:

$$\log_{10}(N) = Rt + \log_{10}(N_0), \text{ where}$$

N = cell count (cells/ml),
R = specific growth rate (day^{-1}),
t = time (days), and
N_0 = initial cell count (10^4 cells/ml).

The median effective concentration was defined as the concentration of tebuthiuron that caused 50% inhibition of the specific growth rate of treated diatom populations. The percent inhibition of specific growth rate at each tebuthiuron concentration was calculated with the following equation:

$$I_R = \frac{R_c - R_t}{R_c} \times 100, \text{ where}$$

I_R = percent inhibition based on specific growth rates,
R_c = mean of the specific growth rates of three replicate control cultures, and
R_t = mean of the specific growth rates of the three replicate cultures at each treatment level.

A linear regression of percent inhibition versus the logarithm of the average analyzed tebuthiuron concentration was used to obtain the median effective concentration. The 95% confidence interval around the regression line was generated using SAS, and a graph of the regression line and associated confidence limits was obtained. The 95% confidence limits for the median effective concentration (EC_{50}) were obtained by graphic interpolation.

12. **REPORTED RESULTS:**

No significant decrease in specific growth rate relative to the control was observed at mean tebuthiuron concentrations $\leq$0.11 mg/L (Table 3; attached). At tebuthiuron concentrations $\geq$0.22 mg/L, specific growth rates were significantly lower than those of the control.

Diatom cell counts on day 7 were not significantly reduced relative to the control at mean tebuthiuron concentrations $\leq$0.11 mg/L, but were significantly

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reduced at concentrations ≥0.22 mg/L (Table 4; attached).

No treatment-related reductions in biomass were observed at average analyzed tebuthiuron concentrations ≤0.056, whereas significant decreases in diatom biomass were observed at concentrations of 0.11, 0.22, 0.46, and 0.89 mg/L. Although a statistically significant decrease in diatom biomass was detected at the 0.0012 mg/L treatment, this change did not appear to be dose-related, as the two higher exposure levels (0.011 and 0.056 mg/L) showed no significant decrease in biomass relative to the control. Based on terminal diatom biomass, the NOEC concentration for tebuthiuron was 0.056 mg/L.

The biomass data from this study suggest that terminal dry weight determination may not be a reliable measure of standing crop for N. pelliculosa. At the three highest tebuthiuron concentrations the measured diatom biomass values were 50% of the control value; however, diatom cell counts (Table 4) indicate that these treatments contained only 1 to 15% as many cells as measured in the control. While the reason for this discrepancy was not firmly established, precipitation of salts from the nutrient medium onto the filter disks used to collect the diatom cells may have interfered with the dry weight determinations.

Using the logarithm of the average analyzed tebuthiuron concentration and the percent inhibition data for tebuthiuron concentrations, a linear regression model estimated the median effective concentration of tebuthiuron to be 0.213 mg/L with 95% confidence limits of 0.155 and 0.282 mg/L (Table 3; attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
Results from this study indicated that the NOEC concentration of tebuthiuron for the freshwater diatom, Navicula pelliculosa, was 0.056 mg/L. Terminal diatom biomass was the most sensitive indicator of toxicity as this parameter was significantly reduced relative to control cultures at tebuthiuron concentrations ≥0.11 mg/L. The median effective concentration (EC_{50}) was determined to be 0.213 mg/L.

A GLP compliance statement was included in the report and the study was audited by Lilly research Laboratories' Quality Assurance Unit. A statement of quality assurance was included in the report, indicating that the study was

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conducted in accordance with U.S. EPA Good Laboratory Practice Standards.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o The maximum label rate was not provided in the report.

o A 25% detrimental effect level was not provided in the study although it was calculated by the reviewer to be 0.111 mg/L.

o The test design states that "each flask was capped with aluminum foil to prevent contamination while allowing free gas exchange". Although the SEP does not state that aluminum foil cannot be used, it seems that aluminum foil would prevent free gas exchange.

B. Statistical Analysis: The reviewer calculated the EC50 value using linear regression by plotting the log of mean measured concentration against the percent inhibition of specific growth rate expressed as probits (attached) and determined it to be 0.193 mg/L rather than 0.213 mg/L calculated by the study authors. The NOEC was calculated by the reviewer using both cell count and biomass and determined to be 0.11 mg/L for both parameters rather than the 0.056 mg/L reported by the study authors.

C. Discussion/Results: The discrepancy in the 7-day EC50 value of tebuthiuron for N. pelliculosa (0.193 vs. 0.213) does not appear substantial. Although the reviewer calculated the NOEC to be 0.11 mg/L rather than 0.056 as reported by the study authors, the lower value is accepted as the most conservative NOEC.

D. Adequacy of the Study:

(1) Classification: Core

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(2) **Rationale:** Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the validity of the toxicity results.

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** Yes, 01-05-90.

Tebuthiuron - Navicula pelliculosa

```
          5. STORE DATA
          6. GO TO PROGRAM MENU
          7. DO ANOTHER REGRESSION
OPTION ? 4
   Navicula
REGRESSION EQUATION:
Y= 7.004395 + 2.80211 X

COEFFICIENT OF CORRELATION= .9455159


PRESS ENTER TO CONTINUE.?
          ACTUAL VERSUS ESTIMATED VALUES
          X=logconc  Y=probit
DATA POINT      X              Y          ESTIMATED Y    ERROR
 1             -2.921          0          -1.180567       1.180567
 2             -1.959          0           1.515062      -1.515062
 3             -1.252          3.36        3.496154      -.1361542
 4             -.959           4.16        4.317172      -.1571722
 5             -.658           5.1         5.160607      -6.060696E-02
 6             -.337           5.52        6.060084      -.5400844
 7             -.051           8.09        6.861487       1.228513
PRESS ENTER TO CONTINUE?
```

EC50

$y = 7.004395 + 2.80211x$

$y = 5.0$

$x = (5.0 - 7.004395)/2.80211$

$x = -0.715$

inv. log = 0.193

EC50 = 0.193 mg/L

EC25

$y = 4.33$

$x = (4.33 - 7.004395)/2.80211$

$x = -0.954$

inv. log = 0.111

EC25 = 0.111 mg/L

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Tebuthiuron - Navicula pelliculosa

NOEC - cell count

Analysis of Variance File: tebnav Date: 01-03-1989

FILTER: None

N's, means and standard deviations based on dependent variable: COUNT

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean - cell count	S.D.
*		24	336.4583	263.2468
1	Control	3	540.0000	74.6659
2	0.0012 mg/L	3	501.6667	88.0814
3	0.011	3	630.0000	190.1973
4	0.056	3	561.6667	123.4234
5	0.11	3	350.0000	112.5833
6	0.22	3	80.0000	18.0278
7	0.46	3	21.6667	7.6376
8	0.89	3	6.6667	2.8868

Fmax for testing homogeneity of between subjects variances: 4341.00
Number of variances= 8 df per variance= 2.

Analysis of Variance Dependent variable: COUNT

Source	df	SS (H)	MSS	F	P
Between Subjects	23	1593874.0000			
C (CONC)	7	1438257.3800	205465.3440	21.125	0.0000
Subj w Groups	16	155616.6250	9726.0391		

JD

NOEC- cell count

Analysis of Variance File: tebnav Date: 01-03-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	540.000	6	80.000
2	501.667	7	21.667
3	630.000	8	6.667
4	561.667		
5	350.000		

Comparison	Tukey-A*	Dunnett
1 > 2		
1 < 3		
1 < 4		
1 > 5		
1 > 6	0.0100	0.0100
1 > 7	0.0100	0.0100
1 > 8	0.0100	0.0100
2 < 3		N.A.
2 < 4		N.A.
2 > 5		N.A.
2 > 6	0.0100	N.A.
2 > 7	0.0100	N.A.
2 > 8	0.0100	N.A.
3 > 4		N.A.
3 > 5	0.0500	N.A.
3 > 6	0.0100	N.A.
3 > 7	0.0100	N.A.
3 > 8	0.0100	N.A.
4 > 5		N.A.
4 > 6	0.0100	N.A.
4 > 7	0.0100	N.A.
4 > 8	0.0100	N.A.
5 > 6		N.A.
5 > 7	0.0500	N.A.
5 > 8	0.0500	N.A.
6 > 7		N.A.
6 > 8		N.A.
7 > 8		N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500). A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

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NOEC - biomass

Analysis of Variance File: tebnav Date: 01-03-1989

FILTER: None

N's, means and standard deviations based on dependent variable: BIOMASS

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean - biomass	S.D.
*		24	0.0125	0.0041
1	control	3	0.0183	0.0042
2	0.0012	3	0.0120	0.0026
3	0.011	3	0.0153	0.0042
4	0.036	3	0.0150	0.0020
5	0.11	3	0.0127	0.0012
6	0.22	3	0.0093	0.0025
7	0.46	3	0.0087	0.0021
8	0.89	3	0.0083	0.0015

Fmax for testing homogeneity of between subjects variances: 13.00
Number of variances= 8 df per variance= 2.

Analysis of Variance Dependent variable: BIOMASS

Source	df	SS (H)	MSS	F	P
Between Subjects	23	0.0004			
C (CONC)	7	0.0003	0.0000	5.180	0.0031
Subj w Groups	16	0.0001	0.0000		

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NOEC - biomass

Analysis of Variance File: tebnav Date: 01-03-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	0.018	6	0.009
2	0.012	7	0.009
3	0.015	8	0.008
4	0.015		
5	0.013		

Comparison	Tukey-A*	Dunnett
1 > 2		
1 > 3		
1 > 4		
1 > 5		
1 > 6	0.0500	0.0100
1 > 7	0.0100	0.0100
1 > 8	0.0100	0.0100
2 < 3		N.A.
2 < 4		N.A.
2 < 5		N.A.
2 > 6		N.A.
2 > 7		N.A.
2 > 8		N.A.
3 > 4		N.A.
3 > 5		N.A.
3 > 6		N.A.
3 > 7		N.A.
3 > 8		N.A.
4 > 5		N.A.
4 > 6		N.A.
4 > 7		N.A.
4 > 8		N.A.
5 > 6		N.A.
5 > 7		N.A.
5 > 8		N.A.
6 > 7		N.A.
6 > 8		N.A.
7 > 8		N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500). A blank means the P-value is greater than 0.0500.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

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FIGURE 2. REGRESSION CURVE USED IN ESTIMATING THE 95% CONFIDENCE LIMITS FOR THE MEDIAN EFFECTIVE CONCENTRATION OF TEBUTHIURON FOR Navicula pelliculosa. STUDY J00886.

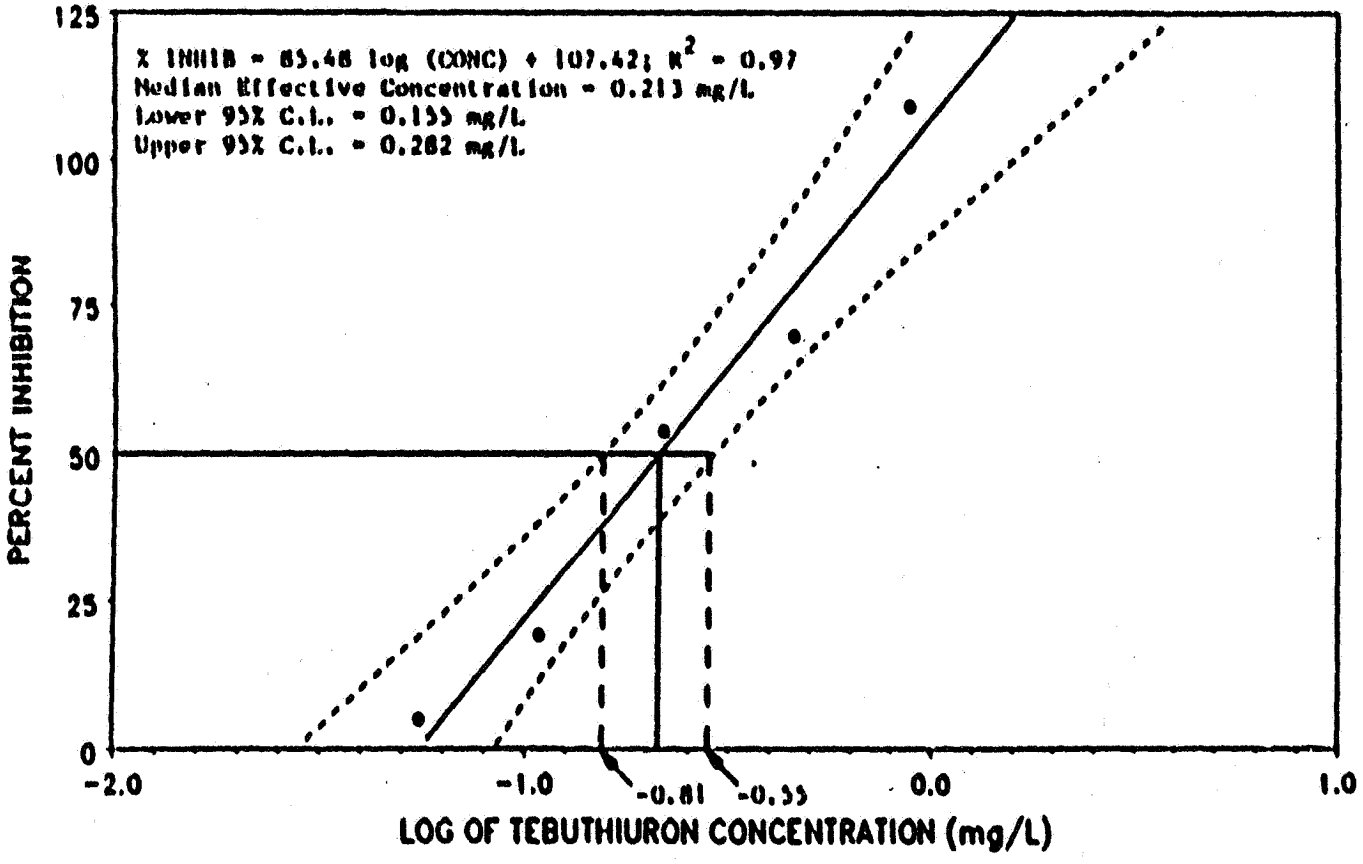

TABLE 3. INHIBITION OF Navicula pelliculosa REPRODUCTION BY TEBUTHIURON DURING THE LOGARITHMIC GROWTH PHASE (DAYS 1 TO 7) AS MEASURED BY SPECIFIC GROWTH RATE. STUDY J00888.

Average Analyzed Tebuthiuron Concentration (mg/L)	Specific Growth Rate[a] (day^{-1})	Percent Inhibition[b]
ND (Control)	0.302 ±0.003	0.0
0.0012	0.304 ±0.044	-0.9
0.011	0.304 ±0.041	-0.8
0.056	0.285 ±0.031	5.4
0.11	0.243 ±0.044	19.6
0.22	0.138* ±0.052	54.1
0.46	0.090* ±0.049	70.2
0.89	-0.029* ±0.053	109.5

* Significantly less than the control ($p \leq 0.05$).

[a] Mean ± SD, n=3. The growth rate of each replicate culture was estimated with the regression equation: $\log_{10}(N) = R \cdot t + \log_{10}(N_0)$, where N = cell count (cells/ml),
R = specific growth rate (day^{-1}),
t = time (days), and
N_0 = initial cell count (10^4 cells/ml).

[b] Calculated by the equation: $I_R = \frac{R_c - R_t}{R_c} \times 100$,

where I_R = percent inhibition based on specific growth rate,
R_c = mean of the specific growth rates of the three replicate control cultures, and
R_t = mean of the specific growth rates of the three replicate cultures at each treatment level.

55-14

TABLE 4. CELL COUNTS AND BIOMASS OF *Navicula pelliculosa* POPULATIONS EXPOSED TO TEBUTHIURON FOR SEVEN DAYS. STUDY J00988.

Average Analyzed Tebuthiuron Concentration (mg/L)	Cell Count On Day 7 (10^3 cells/ml)	Diatom Biomass[a] On Day 7 (mg/ml)
ND (Control)	540 ±75	0.018 ±0.004
0.0012	502 ±88	0.012* ±0.002
0.011	630 ±190	0.016 ±0.004
0.056	562 ±123	0.015 ±0.002
0.11	350 ±113	0.012* ±0.001
0.22	80* ±18	0.009* ±0.003
0.46	22* ±8	0.009* ±0.002
0.89	7* ±3	0.009* ±0.001

* Significantly less than the control ($p \leq 0.05$).

[a] Mean ± SD, n=3. Measured as dry weight of diatom cells.

JE 15

Shaughnessey No. 105501 Chemical Name Tebuthiuron Chemical Class ____ Page ____ of ____

Study/Species/Lab/Accession	Chemical % a.i.	Results	Reviewer/Date	Valid Study
14-Day Single Dose Oral LD_{50}		LD50 = . mg/kg (95% C.L.) Contr. Mort.(%) =		
Species ____		Slope = # Animals/Level = Age(Days) = Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%) =		
Species ____		Slope = # Animals/Level = Age(Days) = Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%) =		
Species		Slope = # Animals/Level = Age(Days) = Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%) =		
Species		Slope = # Animals/Level = Age(Days) = Sex =		
Lab ____		8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%) = Sol. Contr. Mort.(%) =		
Species		Slope = # Animals/Level = Temperature =		
Lab ____		48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
~~96-Hour LC_{50}~~ 7-Day EC50		EC LC50 = 0.193 ppm (95% C.L.) Con. Mort.(%) = N/A	D.S. 1-5-90	Core
Species Daricula pelliculosa 99.08		Slope = 85.48 # Animals/Level = 10,000 Sol. Con. Mor.(%) = N/A		
Lab Eli Lilly		~~96-Hour~~ 7-day Dose Level pp /(% inhibition) Temp. = 24°C		
Acc. 410804-03		0.0012 (-99), 0.011 (-0.8), 0.056 (5.4), 0.11 (19.6), 0.22 (54.1) 0.46 (70.2), 0.89 (109		
		Comments: Based on mean measured concentrations		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%) = Sol. Con. Mort.(%) =		
Species		Slope = # Animals/Level = Temp. =		
Lab ____		96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		

lewis tebuthiuron navicula 5-day mean cell counts

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.39	100	93	93	0
.46	100	90	90	0
.22	100	79	79	0
.11	100	72	72	0
.056	100	41	41	0
.011	100	0	0	0
.0012	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 6.785555E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	3.632219E-02		7.291239E-02	
5.808836E-02		9.041556E-02		

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1265597	3.883811	
1.605892E-03			

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.836442
95 PERCENT CONFIDENCE LIMITS = 1.183124 AND 2.489761

LC50 = 8.135473E-02 0.08135 ms/L LC25 0.0349
95 PERCENT CONFIDENCE LIMITS = 4.896101E-02 AND .1221135

LC10 = 1.655115E-02
95 PERCENT CONFIDENCE LIMITS = 5.064382E-03 AND 3.080127E-02

lewis tebuthiuron Navicula 7-day mean cell counts

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.89	100	99	99	0
.46	100	96	96	0
.22	100	85	85	0
.11	100	35	35	0
.056	100	0	0	0
.011	100	0	0	0
.0012	100	7	7	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1337974

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	1.339839E-02	.1156757	9.711592E-02	.1377568

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
9	13.75014	406.3581	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.764474
95 PERCENT CONFIDENCE LIMITS =-4.778404 AND 8.307351

LC50 = .1128533 mg/L EC25 = 0.0468

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 2.151557E-02
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

TITLE: navicula 5-day
FILE: a:\navic
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	0	1	135.0000	135.0000
1	0	2	215.0000	215.0000
1	0	3	190.0000	190.0000
2	.0012	1	265.0000	265.0000
2	.0012	2	195.0000	195.0000
2	.0012	3	145.0000	145.0000
3	.0011	1	200.0000	200.0000
3	.0011	2	200.0000	200.0000
3	.0011	3	300.0000	300.0000
4	.056	1	115.0000	115.0000
4	.056	2	120.0000	120.0000
4	.056	3	85.0000	85.0000
5	.11	1	70.0000	70.0000
5	.11	2	55.0000	55.0000
5	.11	3	25.0000	25.0000
6	.22	1	55.0000	55.0000
6	.22	2	25.0000	25.0000
6	.22	3	30.0000	30.0000
7	.46	1	10.0000	10.0000
7	.46	2	15.0000	15.0000
7	.46	3	30.0000	30.0000
8	.89	1	5.0000	5.0000
8	.89	2	30.0000	30.0000
8	.89	3	5.0000	5.0000

navicula 5-day
File: a:\navic Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0	3	135.000	215.000	180.000
2	.0012	3	145.000	265.000	201.667
3	.0011	3	200.000	300.000	233.333
4	.056	3	85.000	120.000	106.667
5	.11	3	25.000	70.000	50.000
6	.22	3	25.000	55.000	36.667
7	.46	3	10.000	30.000	18.333
8	.89	3	5.000	30.000	13.333

navicula 5-day
File: a:\navic Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM

navicula 5-day
File: a:\navic Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0	1675.000	40.927	23.629
2	.0012	3633.333	60.277	34.801
3	.0011	3333.333	57.735	33.333
4	.056	358.333	18.930	10.929
5	.11	525.000	22.913	13.229
6	.22	258.333	16.073	9.280
7	.46	108.333	10.408	6.009
8	.89	208.333	14.434	8.333

navicula 5-day
File: a:\navic Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	165150.000	23592.857	18.687
Within (Error)	16	20200.000	1262.500	
Total	23	185350.000		

Critical F value = 2.66 (0.05,7,16)
Since F > Critical F REJECT Ho:All groups equal

navicula 5-day
File: a:\navic Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	180.000	180.000		
2	.0012	201.667	201.667	-0.747	
3	.0011	233.333	233.333	-1.838	
4	.056	106.667	106.667	2.528	
5	.11	50.000	50.000	4.481	*
6	.22	36.667	36.667	4.941	*
7	.46	18.333	18.333	5.573	*
8	.89	13.333	13.333	5.745	*

Dunnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

navicula 5-day
File: a:\navic Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	180.000	180.000		
2	.0012	201.667	201.667	-0.747	
3	.0011	233.333	233.333	-1.838	
4	.056	106.667	106.667	2.528	
5	.11	50.000	50.000	4.481	*
6	.22	36.667	36.667	4.941	*
7	.46	18.333	18.333	5.573	*
8	.89	13.333	13.333	5.745	*

Dunnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

navicula 5-day
File: a:\navic Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	3			
2	.0012	3	74.269	41.3	-21.667
3	.0011	3	74.269	41.3	-53.333
4	.056	3	74.269	41.3	73.333
5	.11	3	74.269	41.3	130.000
6	.22	3	74.269	41.3	143.333
7	.46	3	74.269	41.3	161.667
8	.89	3	74.269	41.3	166.667

slope = 3.493554E-02 1.836442 LC50 = .08135 LC25 = 0.0349

slope = 4.681856E-02 1.764474 LC50 = .1128533 LC25 = 0.0468

F.k

MRID No. 410804-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Tebuthiuron.
Shaughnessey No. 105501.

2. **TEST MATERIAL:** Technical tebuthiuron (EL-103, compound 75503); chemical name: N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea; Lot No. 729-AS7; 99.08% active ingredient.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Lemna gibba.

4. **CITATION:** Negilski, D.S. and P.J. Cocke. 1989. Toxicity of Tebuthiuron to Duckweed (Lemna gibba) in a Static Renewal Test System. Laboratory Project No. J00588. Conducted by Lilly Research Laboratories, Greenfield, Indiana. Submitted by Elanco Products Company, MRID No. 410804-04.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: January 10, 1990
Cheryl A. Sutton 3/22/90

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 1-10-90

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 5/25/90

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier-2 growth and reproduction test using an aquatic macrophyte. Based on the day-14 biomass, the EC25 and EC50 values of tebuthiuron for Lemna gibba were 0.066 and 0.135 mg/L mean measured concentrations, respectively. Based on the frond counts, plant counts, and biomass on day 14 and the calculated EC25, the NOEC value was determined to be <0.066 mg/L mean measured concentration.

8. **RECOMMENDATIONS:** N/A.

9 hrs.

Cy

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** Lemna gibba G-3 used in this test were obtained from stock cultures maintained at the testing facility. The plants were derived from an initial clone provided by Dr. Elaine Tobin of the Biology Department at the University of California at Los Angeles. Stock cultures were grown in a nutrient medium and housed in an environmental growth chamber. The cultures were held at about 25°C and continuously illuminated at approximately 5 klux.

The aqueous nutrient medium used in maintaining stock cultures and testing was the E medium described by Cleland and Briggs (1967). The E medium is equivalent to the M medium of Hillman (1961) modified by the addition of EDTA. The composition of the medium is included in the report. Sucrose was noted as not being included in the medium. The pH of the medium was adjusted to approximately 5.0 using KOH or HCl.

B. **Dosage:** Fourteen-day growth and reproduction test.

C. **Test System and Design:** Based on a pilot study, seven nominal concentrations of tebuthiuron (0.005, 0.01, 0.05, 0.1, 0.2, 0.4, and 0.8 mg/L) and a control were employed in the definitive test. Three replicates were included at each treatment level and the control. To ensure the nutrient availability and to maintain stable tebuthiuron exposure concentrations, the test solution in each vessel was renewed on days 4, 8, and 11.

Test vessels were 600-ml beakers, each containing 300 ml of an appropriate test solution. Initially, a sheet of clear Plexiglas (1/8-inch thick) was placed over the test vessels in an effort to retard evaporation of the solutions. However, by test day 4, approximately 30% of each test solution had evaporated. This problem was remedied by replacing the Plexiglas with the bottom half of a plastic Petri dish. Evaporation was negligible over the remainder of the study.

The test was initiated when three 3-frond plants were randomly distributed to each beaker. The plants floated on the surface of each solution. At test initiation, each vessel was randomly assigned a position in the growth chamber. On each successive test day, the vessels were systematically moved one position in the growth chamber. The temperature and lighting conditions employed during the test were the same as those used for culturing.

The number of fronds and plants in each replicate vessel was counted on days 2, 4, 7, 9, 11, and 14. Every frond visibly projecting beyond the edge of the parent frond was counted. The dry weight of the plants in each vessel was determined on day 14 to obtain a direct measure of the duckweed biomass.

At test initiation and on solution-renewal days (days 4, 8, and 11), samples of the fresh (new) test solutions were collected. The old test solutions were collected on days 4, 8, 11, and at test termination by pooling approximately 33-ml aliquots from the three replicates at each treatment level. All samples were analyzed for tebuthiuron, using high performance liquid chromatography (HPLC).

The temperature and pH of each new test solution were measured at test initiation and on renewal days. The measurements were also performed in each old solution on renewal days and at test termination. Total alkalinity, total hardness, and conductivity of the aqueous nutrient medium were determined at test initiation.

E. **Statistics:** To determine the no-observed-effect concentration (NOEC), treatment responses (i.e., specific growth rates, frond and plant counts from day 14, and biomass on day 14) were compared to the control responses using a one-tailed Dunnett's t-test. The specific growth rate of each replicate culture was determined as the slope of the growth curve (frond count versus time) during the logarithmic phase using the following equation:

$$\log (N) = (R \times t) + \log (N_0)$$

where: N = frond count,
R = specific growth rate (day^{-1}),
t = time (days), and
N_0 = initial frond count (9 fronds).

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The percent inhibition of specific growth rate at each tebuthiuron concentration was calculated using the following equation:

$$I_R = \frac{R_c - R_t}{R_c} \times 100$$

where: I_R = percent inhibition based on specific growth rate,
R_c = mean of the specific growth rates of the three-replicate control cultures, and
R_t = mean of the specific growth rates of the three-replicate cultures at each treatment level.

The median effective concentration (EC50) and its corresponding 95% confidence limits were determined by using a linear regression of percent inhibition versus the logarithm of mean measured concentrations using SAS program.

12. **REPORTED RESULTS:** During the test, the temperature remained between 22.8 and 26.1°C in all solutions. The pH of new and old test solutions ranged from 4.6 to 5.0 and 4.5 to 5.9, respectively. The total hardness, total alkalinity, and conductivity of the nutrient medium at test initiation were 530 mg/L as $CaCO_3$, 5 mg/L as $CaCO_3$, and 605 uS/cm, respectively.

The tebuthiuron concentration at each treatment remained relatively stable over the 7-day test period. The mean measured concentrations were 0.0050, 0.0096, 0.049, 0.091, 0.19, 0.38, and 0.78 mg/L, representing 91 to 100% of the nominal values. On test day 4, increases (18 to 42%) in the concentration of tebuthiuron were measured at all treatment levels (Table 2, attached), resulting from excessive evaporation of the test solution over the first four days of the study when the test vessels were loosely covered with a sheet of clear Plexiglas.

Table 3 (attached) presents specific growth rates for the control and each treatment level. The mean frond count in the control cultures increased from the inoculation level of 9 to 734 on test day 14. This period of rapid vegetative reproduction was considered to represent the logarithmic phase and was used to determine the specific growth rate for each replicate culture. No significant decrease in specific growth rate relative to the control was observed at mean

measured concentrations of ≤0.091 mg/L. Specific growth rates at test concentrations of ≥0.19 mg/L were significantly ($p \leq 0.05$) lower than those of the control. Based on the mean specific growth rate, the NOEC for tebuthiuron was 0.091 mg/L.

Mean frond and plant counts of the duckweed at test termination (day 7) are presented in Table 4 (attached). At mean measured concentrations of ≤0.091 mg/L, the cell counts on day 7 were not significantly reduced when compared to those in the control. Significant reductions in frond and plant counts were found between the control values and those at concentrations of ≥0.19 mg/L. Based on the frond and plant counts at test termination, the NOEC for tebuthiuron was 0.091 mg/L.

Biomass measurements at each test level at test termination are summarized in Table 4 (attached). No significant reductions in biomass were observed at test concentrations of ≤0.091 mg/L when compared to the control. Significant decreases in biomass were found at test concentrations of ≥0.19 mg/L. Based on the duckweed biomass, the NOEC was 0.091 mg/L.

The calculated values of growth inhibition for the control and each treatment were shown in Table 3 (attached). The percentage inhibition data at test concentrations of ≥0.049 were used to calculate the EC50 value. The EC50 determined from a regression analysis was 0.235 mg/L with a 95% confidence interval of 0.151 and 0.389 mg/L. The slope of the regression line and the y-intercept were 84.10 and 102.96, respectively. The coefficient of determination (R^2) was 0.95.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
The NOEC of tebuthiuron for L. gibba was 0.091 mg/L. When compared to the control cultures, specific growth rates, frond and plant counts at test termination, and biomass were significantly reduced at test concentrations of ≥0.19 mg/L. Using the specific growth rate during the logarithmic phase of reproduction as an indicator of the duckweed growth, the EC50 value with 95% confidence limits was 0.235 (0.151-0.389) mg/L. The slope of the dose-response curve was 84.10.

Several inspections had been conducted during the course of the study by the Quality Assurance Unit of Lilly Research Laboratories for compliance with the OECD GLP standards. A GLP statement was included in the report.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o The maximum label rate was not provided in the report. Therefore, it could not be determined if the concentrations tested were less than the maximum label rate as though it were applied directly to the surface of a 15-cm water column.

o Only three plants per replicate were used. The SEP recommends the use of five plants per replicate for Lemna.

o The EC25 value was not reported.

B. Statistical Analysis: The reviewer calculated EC50 and EC25 values for each growth parameter using a regression analysis. All calculations are attached. The EC50 value based on specific growth rate (0.234 mg/L) was similar to that calculated by the author (0.235 mg/L). However, the estimation based on the biomass yielded the lowest EC50 and EC25 (i.e., 0.135 and 0.066 mg/L, respectively). Therefore, these values should be used in the risk assessment of tebuthiuron.

Analysis of variance (ANOVA) with a multiple comparison (Dunnett's) test was performed on the day-14 frond counts, day-14 plant counts, and day-14 biomass to compare the values at each treatment level to those of the control. The printouts are attached. The results confirmed the analyses performed by the authors. Test concentrations of $\geq$0.19 mg/L significantly ($p = 0.01$) decreased the frond counts, plant counts, and biomass of L. gibba when compared to the control values. Since the raw data on specific growth rate were not submitted, the ANOVA on this parameter could not be verified.

C. Discussion/Results: This study is scientifically sound. Based on the biomass on day 14, the EC25 and EC50 values of tebuthiuron for L. gibba were 0.066 and 0.135 mg/L mean measured concentrations, respectively. Based on the decrease in frond counts, plant counts, and biomass at tebuthiuron test concentrations of $\geq$0.19 mg/L and the calculated EC25 above, the NOEC was determined to be <0.066 mg/L.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, January 10, 1990.

20

Technical

Sh. No. 105501 Chemical Name Tebuthiuron Chemical Class ____ Page 1 of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validati Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =		
Lab	____	48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
~~96-Hour~~ 14-Day EC_{50}		* 95% C.L. Based on 14-day biomass EC50 = 0.135 ppm ( ) Con. Mor(%)= N/A		
Species Lemna gibba		** Slope= 79.44 # Animals/Level= 9 Sol. Con. Mor.(%)= N/A	PK 1-10-90	Core
Lab Lilly Research Laboratories 99.08		~~96-Hour~~ 14-Day Dose Level ppm/(%Mortality) Inhibition Temp.= 25°C 0.049(14), 0.09(24), 0.19(77), 0.38(96), 0.78(98)		
Acc. HRID 410804-04		Comments: * Mean measured concentrations, ** X = log conc. Y = % inhibition a = 118.99		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		

TABLE 2. ANALYZED CONCENTRATIONS OF TEBUTHIURON IN THE TEST SOLUTIONS DURING A 14-DAY EXPOSURE OF Lemna gibba. STUDY J00588.

Test Day	Sample	Analyzed Tebuthiuron Concentration (mg/L)							
		0.0 (Control)	0.005	0.01	0.05	0.1	0.2	0.4	0.8
0	New[a]	ND[c]	0.0048	0.011	0.048	0.10	0.20	0.41	0.84
4	Old[b]	ND	0.0062	0.013	0.068	0.13	0.26	0.51	1.01
	New	ND	0.0039	0.0082	0.041	0.083	0.19	0.37	0.78
8	Old	ND	0.0034	0.0074	0.034	0.069	0.16	0.29	0.64
	New	ND	0.0060	0.010	0.054	0.088	0.20	0.41	0.83
11	Old	ND	0.0050	0.0083	0.047	0.076	0.16	0.33	0.65
	New	ND	0.0056	0.010	0.055	0.098	0.20	0.39	0.84
14	Old	ND	0.0049	0.0089	0.048	0.082	0.16	0.32	0.63
Mean ± SD		ND	0.0050 ±0.0010	0.0096 ±0.0018	0.049 ±0.010	0.091 ±0.019	0.19 ±0.034	0.38 ±0.069	0.78 ±0.13

[a] "New" refers to samples collected from treatment stock solutions used to renew the test solutions.

[b] "Old" refers to samples collected by pooling aliquots from the three replicates at each treatment prior to renewal.

[c] ND = None detected (i.e., <0.0005 mg/L).

TABLE 3. INHIBITION OF Lemna gibba REPRODUCTION BY TEBUTHIURON AS MEASURED BY SPECIFIC GROWTH RATE. STUDY J00588.

Log Conc.	Average Analyzed Tebuthiuron Concentration (mg/L)	Specific Growth Rate[a] (1/day)	Percent Inhibition[b]
	ND (Control)	0.149 ±0.003	0.0
−2.3010	0.0050	0.145 ±0.009	2.7
−2.0177	0.0096	0.148 ±0.011	0.9
−1.3098	0.049	0.145 ±0.008	2.9
−1.0410	0.091	0.137 ±0.004	8.3
−0.7212	0.19	0.104* ±0.008	30.4
−0.4202	0.38	0.039* ±0.013	73.7
−0.1079	0.78	0.005* ±0.002	96.9

* Significantly less than the control ($p \leq 0.05$).

[a] Mean ± SD, n=3. The growth rate of each replicate culture was estimated with the regression equation: $\log_{10}(N) = R \cdot t + \log_{10}(N_0)$, where N = frond count,
R = specific growth rate (day^{-1}),
t = time (days), and
N_0 = initial frond count (nine).

[b] Calculated by the equation: $I_R = \frac{R_c - R_t}{R_c} \times 100$,

where I_R = percent inhibition based on specific growth rate,
R_c = mean of the specific growth rates of the three replicate control cultures, and
R_t = mean of the specific growth rates of the three replicate cultures at each treatment level.

TABLE 4. FROND COUNTS, PLANT COUNTS, AND BIOMASS OF Lemna gibba POPULATIONS EXPOSED TO TEBUTHIURON FOR 14 DAYS. STUDY J00588.

Log conc	Average Analyzed Tebuthiuron Concentration (mg/L)	Duckweed[a] Plant Count On Day 14	Duckweed[a] Frond Count On Day 14	% I	Duckweed[b] Biomass On Day 14 (mg)	% I
	ND (Control)	169.3 ±15.7	734.3 ±17.0		85.4 ±1.9	
-2.3010	0.0050	140.7 ±35.7	777.3 ±110.0	-6	103.5 ±19.8	-21
-2.0177	0.0096	166.7 ±50.1	796.3 ±258.0	-8	96.9 ±46.8	-13
-1.3098	0.049	125.0 ±49.3	766.7 ±190.1	-4	97.7 ±23.2	14
-1.0410	0.091	118.0 ±29.3	592.3 ±70.2	19	65.0 ±8.3	24
-0.7212	0.19	47.3* ±11.0	273.0* ±48.4	63	20.0* ±4.9	77
-0.4202	0.38	11.3* ±4.0	31.7* ±10.5	96	3.4* ±1.7	96
-0.1079	0.78	5.3* ±0.6	11.3* ±1.2	98	1.7* ±0.3	98

* Significantly different from the control ($p \leq 0.05$).

[a] Mean ± SD, n=3.

[b] Mean ± SD, n=3. Measured as dry weight of duckweed plants.

Lemna gibba

Specific growth rate

REGRESSION EQUATION:
Y= 103.111 + 84.31854 X

COEFFICIENT OF CORRELATION= .9733929

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=PERCENT INHIBITION (GROWTH RATE)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	-1.3098	3	-7.329392	10.32939
2	-1.041	8	15.33543	-7.335434
3	-.7212	30	42.30051	-12.30051
4	-.4202	74	67.68039	6.319611
5	-.1079	97	94.01307	2.986931

EC_{25} = 0.118 mg/L
EC_{50} = 0.234 "

Frond counts

REGRESSION EQUATION:
Y= 118.9164 + 87.17873 X

COEFFICIENT OF CORRELATION= .943228

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=PERCENT INHIBITION (Frond Counts)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	-1.041	19	28.1633	-9.1633
2	-.7212	63	56.04306	6.956944
3	-.4202	96	82.28385	13.71615
4	-.1079	98	109.5098	-11.50977

EC_{25} = 0.084 mg/L
EC_{50} = 0.162 "

L. gibba

Biomass

REGRESSION EQUATION:
Y= 118.9949 + 79.4351 X

COEFFICIENT OF CORRELATION= .948202

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=PERCENT INHIBITION (BIOMASS)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	-1.3098	14	14.95075	-.9507523
2	-1.041	24	36.30291	-12.30291
3	-.7212	77	61.70626	15.29374
4	-.4202	96	85.61623	10.38377
5	-.1079	98	110.4238	-12.42381

EC_{25} = 0.066 mg/L
EC_{50} = 0.135 "

Analysis of Variance File: TEBUT3 Date: 01-08-1989

FILTER: None

N's, means and standard deviations based on dependent variable: FCOUNT

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean	S.D.
*		24	493.3750	344.1200
1	Control	3	734.3333	17.0392
2	0.005 mg/L	3	777.3333	109.9651
3	0.0096 "	3	796.3333	257.9890
4	0.049 "	3	766.6667	190.1166
5	0.091 "	3	592.3333	70.2306
6	0.19 "	3	237.0000	48.4458
7	0.38 "	3	31.6667	10.5040
8	0.78 "	3	11.3333	1.1547

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Fmax for testing homogeneity of between subjects variances: 49918.75

Number of variances= 8 df per variance= 2.

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Analysis of Variance Dependent variable: FCOUNT

Source	df	SS (H)	MSS	F	P
Between Subjects	23	2723627.5000			
C (CONC)	7	2478675.0000	354096.4400	23.129	0.0000
Subj w Groups	16	244952.5000	15309.5312		

Analysis of Variance File: TEBUT3 Date: 01-08-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	734.333	6	237.000
2	777.333	7	31.667
3	796.333	8	11.333
4	766.667		
5	592.333		

Comparison	Dunnett		
1 < 2			
1 < 3			
1 < 4			
1 > 5			
1 > 6	0.0100	*	0.19 mg/L
1 > 7	0.0100	*	0.38 "
1 > 8	0.0100	*	0.78 "
2 < 3	N.A.		
2 > 4	N.A.		
2 > 5	N.A.		
2 > 6	N.A.		
2 > 7	N.A.		
2 > 8	N.A.		
3 > 4	N.A.		
3 > 5	N.A.		
3 > 6	N.A.		
3 > 7	N.A.		
3 > 8	N.A.		
4 > 5	N.A.		
4 > 6	N.A.		
4 > 7	N.A.		
4 > 8	N.A.		
5 > 6	N.A.		
5 > 7	N.A.		
5 > 8	N.A.		
6 > 7	N.A.		
6 > 8	N.A.		
7 > 8	N.A.		

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Analysis of Variance File: TEBUT4 Date: 01-08-1989

FILTER: None

N's, means and standard deviations based on dependent variable: PCOUNT

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean	S.D.
*		24	97.9583	68.8972
1	Control	3	169.3333	15.6950
2	0.005 mg/L	3	140.6667	35.7258
3	0.0096 "	3	166.6667	50.1431
4	0.049 "	3	125.0000	49.2747
5	0.091 "	3	118.0000	29.3087
6	0.19 "	3	47.3333	10.9697
7	0.38 "	3	11.3333	4.0415
8	0.78 "	3	5.3333	0.5774

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Fmax for testing homogeneity of between subjects variances: 7543.00

Number of variances= 8 df per variance= 2.

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Analysis of Variance Dependent variable: PCOUNT

Source	df	SS (H)	MSS	F	P
Between Subjects	23	109176.9530			
C (CONC)	7	94254.9610	13464.9941	14.438	0.0000
Subj w Groups	16	14921.9922	932.6245		

Analysis of Variance File: TEBUT4 Date: 01-08-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	169.333	6	47.333
2	140.667	7	11.333
3	166.667	8	5.333
4	125.000		
5	118.000		

Comparison	Dunnett	
1 > 2		
1 > 3		
1 > 4		
1 > 5		
1 > 6	0.0100 *	0.19 mg/L
1 > 7	0.0100 *	0.38 "
1 > 8	0.0100 *	0.78 "
2 < 3	N.A.	
2 > 4	N.A.	
2 > 5	N.A.	
2 > 6	N.A.	
2 > 7	N.A.	
2 > 8	N.A.	
3 > 4	N.A.	
3 > 5	N.A.	
3 > 6	N.A.	
3 > 7	N.A.	
3 > 8	N.A.	
4 > 5	N.A.	
4 > 6	N.A.	
4 > 7	N.A.	
4 > 8	N.A.	
5 > 6	N.A.	
5 > 7	N.A.	
5 > 8	N.A.	
6 > 7	N.A.	
6 > 8	N.A.	
7 > 8	N.A.	

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Analysis of Variance File: TEBUT5 Date: 01-08-1989

FILTER: None

N's, means and standard deviations based on dependent variable: BIOMASS

* Indicates statistics are collapsed over this factor

Factors: C		N	Mean	S.D.
*		24	59.2125	45.2318
1	control	3	85.4000	1.8520
2	0.005 mg/L	3	103.5000	19.7919
3	0.0096 "	3	96.9333	46.8504
4	0.049 "	3	97.7333	23.1865
5	0.091 "	3	64.9333	8.2978
6	0.19 "	3	20.0333	4.8439
7	0.38 "	3	3.4333	1.6503
8	0.78 "	3	1.7333	0.3055

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Fmax for testing homogeneity of between subjects variances:23517.47

Number of variances= 8 df per variance= 2.

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Analysis of Variance Dependent variable: BIOMASS

Source	df	SS (H)	MSS	F	P
Between Subjects	23	47056.0820			
C (CONC)	7	40610.3670	5801.4810	14.401	0.0000
Subj w Groups	16	6445.7148	402.8572		

Analysis of Variance File: TEBUT5 Date: 01-08-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	85.400	6	20.033
2	103.500	7	3.433
3	96.933	8	1.733
4	97.733		
5	64.933		

Comparison	Dunnett	
1 < 2		
1 < 3		
1 < 4		
1 > 5		
1 > 6	0.0100 *	0.19 mg/L
1 > 7	0.0100 *	0.38 "
1 > 8	0.0100 *	0.78 "
2 > 3	N.A.	
2 > 4	N.A.	
2 > 5	N.A.	
2 > 6	N.A.	
2 > 7	N.A.	
2 > 8	N.A.	
3 < 4	N.A.	
3 > 5	N.A.	
3 > 6	N.A.	
3 > 7	N.A.	
3 > 8	N.A.	
4 > 5	N.A.	
4 > 6	N.A.	
4 > 7	N.A.	
4 > 8	N.A.	
5 > 6	N.A.	
5 > 7	N.A.	
5 > 8	N.A.	
6 > 7	N.A.	
6 > 8	N.A.	
7 > 8	N.A.	

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

lewis tebuthiuron lemna frond count

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.78	100	98	98	0
.38	100	96	96	0
.19	100	63	63	0
.091	100	19	19	0
.049	100	0	0	0
9.600001E-03		100	0	0
0				
.005	100	0	0	0

THE BINOMIAL TEST SHOWS THAT .091 AND .19 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1543143

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	1.618376E-02	.162095	.1398908	.1889315

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.101908	2.780902	1.622689E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.998358
95 PERCENT CONFIDENCE LIMITS = 2.721961 AND 5.274755

LC50 = .1586298
95 PERCENT CONFIDENCE LIMITS = .1281473 AND .1966771

LC10 = .0763404
95 PERCENT CONFIDENCE LIMITS = 5.030507E-02 AND 9.828933E-02

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